Observations on the Amœbæ in the Intestines of Persons Suffering from Goitre in Gilgit.

By

Robert McCarrison, M.D., M.R.C.P.Lond.,
Captain Indian Medical Service.

With 24 Text-figures.

My researches on the ætiology of endemic goitre have led me to the conclusion that in all probability the organism which is responsible for the production of this disease is to be found in the intestinal tract. It is necessary, therefore, to give some account of the Protozoa which are so frequently present in this situation. This paper deals only with the organisms of the Amœba group, and simply gives a brief description and figures of the amœbæ found. It does not claim to be a complete account of their life-histories. While no definite statement can be made as to the pathogenicity of these amœbæ, their possible importance is obvious when it is remembered that goitre is due to an organism carried by water.

The examination of the fresh fæces of goitrous individuals was undertaken solely for the purpose of determining the presence or absence of Protozoa. This object was found to be greatly facilitated by the addition of a small quantity of iodine-water to the specimen, which caused the amœbæ to stand out clearly from surrounding objects. One hundred and three cases were examined in this way; amœbæ were present in eighty-seven. In forty-eight cases they were found in large numbers, in twenty-seven in moderate numbers, and in twelve only after considerable time had been spent in

searching for them. The fæces of 101 non-goitrous individuals, living in the same locality, have also been examined. Amœbæ were present in twenty-nine of these; the infection was plentiful in eight, moderate in nine, and scanty in twelve. The typical cysts hereafter described were also found in the only case of goitre from another locality which I have examined.

Two distinct amœbæ are found in the fæces; these have not been distinguished in the live state:

(1) A free amœba which proceeds to encyst and develop into a typical 8-nucleated cyst.

TEXT-FIG. 1.[1]

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TEXT-FIG. 2.

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Text-fig. 1.—Amœba I. Encysted amœba, showing single nucleus and port-wine staining area.

Text-fig. 2.—Amœba I. Encysted amœba. Shows the kidney shape of the port-wine staining area frequently observed.

(2) A free amœba which does not form obvious cysts, but multiplies by division and budding.

In addition, a third amœboid body, enclosed in a characteristic capsule, is also present. Its affinities are not clear, and I can only note its occurrence.

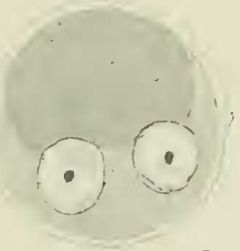
[1] [Figs. 1–7 and 23 are drawn from freehand sketches of the living animals. The preparations were treated with iodine water. Drawings made under Leitz $\frac{1}{12}$ in. oil-immersion, ocular No. 4.

The remaining figures were drawn from fixed and stained preparations, under Zeiss 3 mm. apochromatic homog. oil-immersion, comp. oc. 12 (× 2000). The drawings were made by Miss Rhodes, to whose skill in so accurately depicting the appearances observed I must pay a tribute. The preparations were stained with Haidenhain's hæmatoxylin and Delafield's hæmatoxylin.]

The Amœbæ in the Fresh State.

(*a*) The free amœbæ.—I can only give a general account of the free amœbæ in the live state, an account which covers both species. In specimens treated with iodine solution the organisms are more or less spherical. Their size varies between 12 μ and 20 μ. Larger forms are occasionally seen. The protoplasm is granular, stains yellow with iodine, and is very rarely vacuolated. A differentiation of the protoplasm into ectoplasm and endoplasm can only be made out in those animals which show pseudopodia, and these are few. The

Text-fig. 3.



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Text-fig. 4.

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Text-fig. 3.—Amœba I. Encysted amœba. Bi-nucleated stage with large port-wine staining area.

Text-fig. 4.—Amœba I. Encysted amœba. A stage not commonly met with, showing four nuclei and centrally placed port-wine staining area.

protoplasm contains food-vacuoles and other inclusions. I have never seen any evidence of the ingestion of blood-corpuscles or of epithelium. The nucleus, where observed, is spherical or oval, and is sometimes surrounded by a narrow halo. In the larger organisms it measured 5 μ–8 μ. The characters of the nucleus are very distinct in the two species; they have been studied only in stained specimens.

(*b*) The encysted forms.—Live cysts of definite contour are very commonly seen; they represent stages in the life-history of Amœba I, and show different appearances dependent on the length of time the animal has been encysted. These cysts are, as a rule, perfectly spherical, but they may be oval.

Their size varies from 14 μ–20 μ, the latter being the almost constant diameter of the 8-nucleated forms. The cyst-wall varies in thickness, and sometimes it is made out with difficulty. It encloses a yellow staining granular protoplasm, one or more nuclei, and a characteristic port-wine staining area when treated with iodine (text-figs. 1 to 4). The nucleus is spherical, very clearly defined, and shows refractile granules on its surface and in its interior. A well-marked karyosome is usually present. The nucleus varies in size from 2 μ–3 μ in the 8-nucleated cysts to 6 μ–8 μ in the single-nucleated

TEXT-FIG. 5.

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TEXT-FIG. 6.

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Text-fig. 5.—Amœba I. Encysted amœba. The nuclear division has resulted in the formation of five nuclei; compare text-fig. 17.

Text-fig. 6.—Amœba I. Encysted amœba, 8-nucleated stage, cyst-wall ill-defined; a centrally placed remnant of the port-wine staining area is seen—a very uncommon appearance at this stage.

form. The nuclei vary from one to eight in number, dependent on the phase of development of the cyst (text-figs. 1 to 7). The commonest phases met with are those where the cyst contains one, two, or eight nuclei. Cysts showing four nuclei are much less commonly found. The port-wine staining area is present in the majority of all cysts containing one to four nuclei (text-figs. 1 to 4). It is not, as a rule, present in the 8-nucleated cyst; I have only met with it at this stage of development in one instance (text-fig. 6). It is oval, spherical, or kidney-shaped in form, and occupies about one half of the

cyst. It usually lies in one hemisphere while the protoplasm is situated in the other, but it may be centrally placed. It appears sometimes to alter its position relative to the nuclei and to the cyst-wall. The port-wine reaction with iodine marks off the area from the rest of the protoplasm in a most distinctive way, for while the protoplasm is granular and stains yellow this area appears to be structureless.

I have never been able to observe a division of the protoplasm around the nuclei in the large 8-nucleated cysts.

TEXT-FIG. 8.

TEXT-FIG. 7.

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TEXT-FIG. 9.

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Text-fig. 7.—Amœba I. Encysted amœba. The nuclear division has resulted in the formation of five nuclei; compare text-fig. 17.

Text-fig. 8.—Amœba I. Free amœba. Sideview of organism showing finely granular appearance of protoplasm. The nucleus is seen surrounded by a narrow halo, and shows chromatin more or less evenly distributed with slight massing at four points of the periphery. A central karyosome is seen.

Text-fig. 9.—Amœba I. Unencysted amœba. Probably a stage in simple fission of free amœba. Chromatin massed at periphery of nuclei, which have beaded appearance. Karyosome is seen; nature of included bodies not known.

(*c*) The third organism referred to is less commonly found. The following are its main characteristics in both fresh and stained specimens. In the living state it is seen as a pear-shaped, oval, or spherical body, having a well-defined clear capsule. The protoplasm is granular and stains yellow with iodine. It is split up by a median fissure, on either side of

which and approximately at right angles to it is a shorter fissure, which further divides up the protoplasm (text-fig. 23). Movements of the protoplasm and alterations in the position of the nuclei within the capsule are sometimes seen. The nuclei, four in number, usually lie clumped together or in the position shown in the figure (text-fig. 23). They are clear, spherical bodies of uniform size, very sharply defined, and sometimes showing a central dot, features which are well brought out by staining (text-fig. 24). When the organism is

TEXT-FIG. 10.

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TEXT-FIG. 11.

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TEXT-FIG. 12.

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Text-fig. 10.—Amœba I. Encysted amœba. Protoplasm filled with spherical hyaline masses of variable size. Two chromatin masses are seen in the protoplasm. Chromatin heaped up at opposite poles of nucleus; karyosome well marked.

Text-fig. 11.—Amœba I. Encysted amœba. Protoplasm shows hyaline masses of larger size. Nucleus has divided into two. Reticular structure of nucleus and karyosome seen.

Text-fig. 12.—Amœba I. Encysted amœba. Protoplasm shows single hyaline mass. Nuclei as in Text-fig. 11, but of larger size.

stained the capsule is not coloured, and it appears to be open at its broader end. The granular protoplasm sometimes stains so deeply that no structure can be made out. In the more faintly stained animals the protoplasmic fissures referred to can be seen, but not with such distinctness as in the living animal (text-fig. 24). Sometimes the protoplasm, with its contents, is seen contracted up at one side of the capsule. The size of this animal in its longest diameter is fairly constant, and measures in the live state 14 μ–15 μ.

The Fixed and Stained Amœbæ.

The material used for this part of the investigation was sent from Gilgit to England in Schaudinn's fixing reagent (saturated watery solution of corrosive sublimate two parts, alcohol one part). The methods of staining employed were Delafield's and Haidenhain's hæmatoxylin. The examination of the material was carried out in Professor Minchin's laboratory at the Lister Institute, to whom I am indebted for help and advice.

Text-fig. 13.

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Text-fig. 14.

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Text-fig. 13.—Amœba I. Encysted amœba. Shows well-marked cyst-wall. A large hyaline mass occupying about one half of the cyst is seen. Chromatin masses lie above and below this area. Nuclei are seen lying at either side of cyst, each surrounded by halo. Note the reticular structure and granular appearance of nuclei. No karyosome is seen in either nucleus.

Text-fig. 14.—Amœba I. Encysted amœba. One nucleus has proceeded to second division before the other. A common appearance of the nuclei —wheel-like—at this stage is well seen. Two chromatin masses in protoplasm.

A study of the stained amœbæ shows that there are two distinct species present. In view of the many opinions held with regard to intestinal amœbæ I hesitate to describe these organisms under specific names. Nevertheless, I am inclined to think that Amœba I, which forms the 8-nucleated cysts, is the Entamœba coli, Schaudinn, and that Amœba II corresponds to the Entamœba histolytica, Schaudinn. Certain points in which they appear to differ from the two

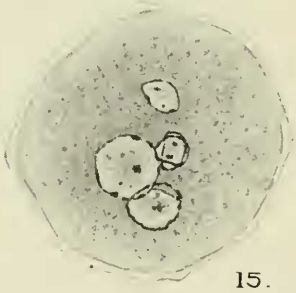
species of Schaudinn will be referred to in the course of my description.

Amœba I.

The unencysted amœba usually appears as a spherical body of variable size, ranging up to 20 μ in diameter. The protoplasm is finely and evenly granular (text-fig. 8). I can detect no differentiation into ectoplasm and endoplasm.

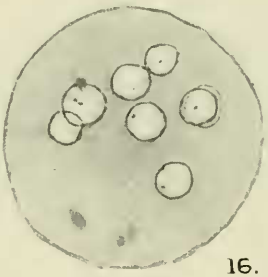
In some the protoplasm contains food-material and various inclusions, while in others it appears to be free from

TEXT-FIG. 15.



15.

TEXT-FIG. 16.



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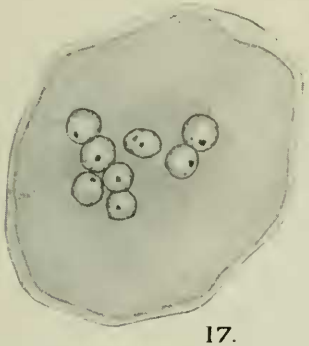
Text-fig. 15.— Amœba I. Encysted amœba. The nuclear division has resulted in the formation of five nuclei.

Text-fig. 16.—Amœba I. Encysted amœba. Typical 8-nucleated cyst, of very common occurrence in fæces. Each nucleus is ring-like with a small karyosome in its interior. A few small chromatin masses are seen.

extraneous matter. Such inclusions as blood-corpuscles or epithelium have never been met with. The protoplasm rarely shows a vacuole. The nucleus is very distinct, and is usually centrally placed (text-fig. 8). It is commonly surrounded by a narrow but distinct halo. In text-fig. 8 the nucleus appears to lie in a cavity lined by a membrane. Since it has been preserved in a sublimate mixture the appearance may be due to shrinkage of the protoplasm. The nucleus stains deeply; it is rich in chromatin, which in the adult unencysted animal is more or less uniformly distributed throughout this structure. There is often a slight tendency for the chromatin to be

massed irregularly at the periphery of the nucleus. It is generally reticulate in character, and, as a rule, shows a distinct karyosome. I have not been able to satisfy myself as to whether division of the nucleus takes place in the unencysted organism or not. Text-fig. 9 represents an organism of a type only occasionally met with, in which it was impossible to make out the existence of a cyst-wall. This is probably a stage in simple fission of the free amœba. Nevertheless I hesitate to offer an opinion on the point, and simply draw attention to the figure. One point is certain, that in all

TEXT-FIG. 17.



17.

TEXT-FIG. 18.

18.

Text-fig. 17.—Amœba I. Encysted amœba; 8-nucleated cyst. The cyst-wall is thicker than in text-fig. 16. The nuclei are very clearly defined and each shows a karyosome.

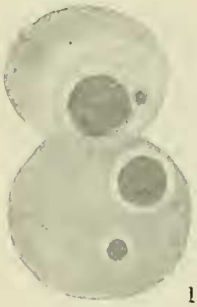
Text-fig. 18.—Amœba I. Encysted amœba. Abnormal form showing twelve nuclei.

organisms in which there were more than two nuclei a cyst-wall was always obvious. Unfortunately I have not noted a similar appearance in the living state, but, as I have said, my observations were then only diagnostic.

The encysted amœba.—The earliest stages of encystment which I have observed are shown in text-fig. 10. The chromatin is heaped up at the periphery of the nucleus, and here and there in the protoplasm dark masses presenting staining reactions similar to those of chromatin are occasionally found. The appearance of the protoplasm is dis-

tinctive; it seems to be filled with spherical hyaline masses of variable size. It is difficult to offer an opinion as to the nature of these spherical masses. Such text-figures as 11 and 12 suggest that they gradually fuse, with the ultimate formation of a large, clear hyaline body, as seen in text-fig. 13. This fusion appears to take place in those stages which represent the division of the nucleus after encystation. I am convinced that the clear area (text-fig. 13) is that which gives rise to the characteristic port-wine reaction with iodine water in the living animal. The hyaline body becomes less marked as

TEXT-FIG. 19.



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TEXT-FIG. 20.

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TEXT-FIG. 21.

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Text-fig. 19.—Amœba II. Two typical organisms. Shows pale staining nuclei surrounded by narrow halo.

Text-fig. 20.—Amœba II. Organism showing three nuclei and two darkly staining bodies. The precise nature of the latter objects is unknown; probably food-material.

Text-fig. 21.—Amœba II. A multi-nucleated organism. Nuclei of varying sizes. A stage of multiple division.

the further development of the organism proceeds to the typical 8-nucleated form; ultimately it completely disappears. This hyaline body is at its highest development in the late bi-nucleated cyst. I regard this spherical mass as being of the nature of food material, a view which is upheld by Jurgens (**2**). A similar appearance has been described by Wenyon (**3**) in Entamœba muris, and he has considered it to be "of the nature of food products which have not been thrown out of the animal." It is questionable, however, whether the body seen by Wenyon is identical with that

which I am describing. Wenyon has found that the refractile body of E. muris "stains feebly, and shows a coarse reticular structure," and that on breaking up "the separate parts shrink to form masses which stain deeply with hæmatoxylin." The hyaline body I have described has not these characters. Schaudinn (4) has described the protoplasm of the encysted E. coli as "divisible into an outer and denser layer containing the nucleus and an inner and more liquid portion,"

TEXT-FIG. 22.

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TEXT-FIG. 23.

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TEXT-FIG. 24.

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Text-fig. 22.—Amœba II. Group of amœbæ, the result of multiple division; a common appearance.

Text-fig. 23.—The third amœboid body. Shows the characteristic capsule and fissured protoplasm as observed in the fresh state. The drawing also shows a common position of the four spherical nuclei.

Text-fig. 24.—The third amœboid body. Shows the typical appearance of this organism when stained. Note the slightly beaded appearance of the nuclei, the presence of karyosomes, the position of the nuclei, and the manner in which they lie clumped together. The unstained capsule and the fissured protoplasm is well seen.

and Wenyon considers that the more liquid portion probably corresponds to the refractile body of E. muris. It does not, however, correspond to the hyaline body of the amœba under consideration. The characteristic port-wine reaction differentiates it from the yellow-staining granular protoplasm. This hyaline body is a conspicuous feature of the great majority

of encysted amœbæ during the earlier phases of their development.

At the time of encystment there is one nucleus present (text-fig. 10). This nucleus divides into two daughter-nuclei (text-figs. 11, 12). I have not been able to trace the complicated series of nuclear changes described by Schaudinn in E. coli and by Wenyon in E. muris as occurring at this stage, though I have seen a large number of amœbæ. The two daughter-nuclei lie most commonly side by side (text-figs. 11, 12). At this stage they vary greatly in size. I have seen them so large their diameter almost equalled half that of the cyst containing them. I have observed in one case a division-figure corresponding closely to fig. 73 of Dobell's paper (**5**); unfortunately the specimen could not be drawn. In text-fig. 13 the two nuclei have separated to either side of the cyst. Division of the two daughter-nuclei takes place, and text-fig. 14 shows that one nucleus has proceeded to the second division before the other. It has been difficult to find examples showing four nuclei; text-fig. 15 shows a further division of the nuclei into five. The ultimate division into eight is shown in text-fig. 16. The cyst-wall eventually becomes thickened as in text-fig. 17. Division of the protoplasm around the nuclei has never been seen. Very rarely a form showing more than eight nuclei is met with (text-fig. 18), but it is so rare that we must regard it as abnormal.

It is evident that the animal here described corresponds very closely to the Entamœba coli of Schaudinn and to the Entamœba muris of Wenyon. The descriptions, however, do not correspond as regards the hyaline body, which is so characteristic of this organism; nor have I been able to trace in it the nuclear changes described by these observers.

Amœba II.

Amœbæ of this species are exceedingly plentiful in some cases; as many as three or four are often found in one field of the microscope. They may occur alone or in association

with one or other of those described in the preceding sections.

The animal is usually spherical and of variable size, the forms most commonly met with averaging 15 μ–20 μ in diameter. The protoplasm is sometimes divisible into a granular endoplasm with a thin layer of ectoplasm surrounding it. The ectoplasm is most obvious in these rare cases in which protrusions in the form of pseudopodia are seen. Often it is not very clearly marked off. The endoplasm is finely granular and contains extraneous matter; such inclusions as blood-corpuscles or epithelium have never been seen. I have not observed vacuoles. The nucleus in some cases is very difficult to see, presenting as it does staining reactions differing only a little from those of the protoplasm. It is frequently surrounded by a clear halo (text-fig. 19). It is very poor in chromatin and does not appear to possess a karyosome. There is nothing distinctive in the position of the nucleus; it may be central, but is more frequently excentric. It varies very considerably in size. Forms are commonly seen containing two or more nuclei (text-figs. 20, 21). Multiplication is apparently very rapid, and takes place either by a process of simple division or by multiple division of the nuclei with budding of the protoplasm, examples of which processes are shown in text-figs. 19 and 22). This organism does not encyst as does Amœba I. I have been unable to find that it develops cysts such as Schaudinn has described in E. histolytica. It is true that I have frequently found small, dark brown, spherical bodies in preparations where this was the only amœba present; their structure could not be made out, nor could it be shown that they had any connection with this amœba.

This organism resembles closely the E. histolytica of Schaudinn. There was, however, no evidence that patients whose fæces swarmed with this Amœba were suffering from dysentery.

I would also like to point out that the amœba here des-

cribed is much smaller in size than the Entamœba histolytica of Schaudinn.

Lister Institute,
March 9th, 1909.

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